

Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi

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Abstract

This work investigates the ability of ericoid mycorrhizal (ErM) and ectomycorrhizal (EcM) fungi to solubilize different toxic metal (Cd, Cu, Pb, Zn)-containing minerals. Minerals were incorporated into solidified agar media and solubilization assessed by measuring clearing of the agar after fungal growth. Measurement of radial growth and biomass dry weight provided indications of metal tolerance: accumulated metal in the biomass was measured by atomic absorption spectrophotometry. Metal tolerance and solubilizing ability varied widely between different mineral and fungal species, and strains derived from sites of differing degrees of metal pollution. Zinc phosphate exhibited the least toxicity and was the easiest to solubilize by the majority of tested fungal isolates. Solubilization of toxic metal minerals was connected with both the pH of the medium and growth and tolerance of fungi and it seems that acidification of the medium was the main mechanism of mineral dissolution for most of the mycorrhizal fungi studied. A very strong lethal effect was observed for ectomycorrhizal isolates (>60% of strains) in the presence of Pb phosphate, carbonate, sulphide and tetraoxide. In contrast, ericoid mycorrhizal isolates were able to grow on Pb-mineral-amended media. A significant proportion of ericoid mycorrhizal cultures (70–90%) solubilized Cd and Cu phosphates and cuprite. None of the ericoid mycorrhizal and ectomycorrhizal fungi were able to produce a clear zone in Pb mineral-containing agar. However, many fungi were able to accumulate mobilized Pb in their mycelia. Differences in toxic metal mineral tolerance, mineral solubilization and metal uptake between populations isolated from metal-polluted and uncontaminated sites were related to the toxic metal which was the main pollutant in the original contaminated environment. In general, metal-tolerant fungi grew and solubilized toxic metal minerals better than non-tolerant isolates.

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1. Introduction

The extent of metal and radionuclide contamination in the world is immense. In the soil environment, metals and radionuclides can be dissolved in solution, held on inorganic soil constituents through various sorption or ion exchange reactions, complexed with soil organic materials, or precipitated as pure or mixed solids (Knox et al., 2000). Unlike degradable organic contaminants and even short-lived radionuclides that can become less toxic over time, metals can be considered conservative because they are not

decomposed in the environment. The influence of micro-biological processes on contamination of the environment by toxic metals and radionuclides is of economic and environmental significance (Gadd, 1993, 2001). However, the potential of microbial processes for bioremediation may be dependent on the physical and chemical nature of the site which influences the form in which metals occur. Furthermore, mineral components contain considerable quantities of metals which are biologically unavailable. Certain microbial processes dissolve metal minerals thereby increasing metal bioavailability and potential toxicity, whereas others immobilize them and reduce bioavailability. As well as being an integral component of biogeochemical cycles for metals and associated elements, these processes

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may be exploited for the treatment of contaminated solid and liquid wastes. Bioremediation, especially if combined with phytoremediation, has been considered as a feasible approach for the remediation of metal-polluted soils (Van der Lelie et al., 2001).

Many fungi can survive and grow in high concentrations of toxic metals (Gadd, 1993). The mechanisms by which they are able to deal with these metals are numerous and varied in their action, e.g. extracellular metal sequestration and precipitation, metal binding to the fungal cell walls, intracellular sequestration and complexation, compartmentation or volatilization (Gadd, 1993). Ecto- and endomycorrhizal symbioses can play a crucial role in protecting plants from toxic metals. The ability of mycorrhizal associations to ameliorate metal toxicity to higher plants has been shown for ericoid mycorrhizas (Bradley et al., 1981), ectomycorrhizas (Brown and Wilkins, 1985a,b; Denny and Wilkins, 1987a,b; Van Tichelen et al., 2001) and arbuscular mycorrhizas (Gildon and Tinker, 1983; Heggo and Angle, 1990). The efficiency of protection, however, differs between distinct isolates of mycorrhizal fungi and different toxic metals and protective effects cannot be demonstrated for all associations in all circumstances (Meharg and Cairney, 2000). What is clear is that plant roots and their associated free-living and symbiotic microbial populations significantly alter the physico-chemical characteristics of the rhizosphere by metabolic activities, resulting in a geochemical environment that can be very different from the bulk soil (Olsson and Wallander, 1998; Whitelaw et al., 1999). This will have significant consequences for the biogeochemical mobility of metals and associated elements in such an environment.

Phosphorus is an essential element for plant and microbial nutrition and can only be assimilated as soluble phosphate species. However, in the soil, a large part of the P pool is poorly soluble. Numerous studies have concluded that an important role of mycorrhizal fungi is to improve plant P nutrition, emphasizing the phosphate-solubilizing ability of mycorrhizal fungi (Lapeyrie et al., 1991; Wallander et al. 1997). Of special interest are the mechanisms by which fungi and plants obtain phosphate since solubilization of inorganic phosphates can result in release of the associated metals (Gadd, 1986). Conversely, formation of insoluble metal phosphates will reduce both metal and phosphate bioavailability. For other insoluble metal compounds and minerals (e.g. various sulphates, oxides, carbonates), solubilization can also result in release of anionic species. The ability of ericoid- and ectomycorrhizal fungi to dissolve and transform Ca-containing insoluble compounds and minerals (phosphates, carbonate and sulphate) in pure culture and in mycorrhizal association has been reported (Callot et al., 1985; Lapeyrie et al., 1991; Gharieb and Gadd, 1999). However, the dissolution of toxic metal-containing minerals increases toxic metal mobility and therefore, possible toxicity to fungi, plants, and other organisms. Processes of toxic metal mineral solubilization

by fungi should therefore be considered in connection with the metal tolerance of these organisms. However, this aspect has been neglected and only a few studies on toxic metal mineral dissolution by ericoid mycorrhizal fungi have been carried out (Martino et al., 2003). Lead, Zn, Ca and Cu are the most frequently identified inorganic contaminants in soil and groundwater (in the same order of their relative occurrence), with Pb being considered particularly dangerous because of its wide distribution and the existence of a variety of Pb-containing minerals (Knox et al., 2000). Therefore, our aims were to study the ability of a range of ericoid- and ectomycorrhizal fungi to solubilize Pb-, Cu-, Cd- and Zn-containing insoluble minerals; to elucidate the mechanisms involved and possible connections with tolerance to solubilized metals, and to compare these processes between ericoid and ectomycorrhizal species from differently polluted habitats.

2. Materials and methods

2.1. Organisms and their origins

We used 12 cultures of the ericoid mycorrhizal fungi *Hymenoscyphus ericae* (provided by Professor A. Meharg) and *Oidiodendron maius* (provided by Dr E. Martino), and 19 cultures of ectomycorrhizal fungi belonging to the genera *Cenococcum*, *Hebeloma*, *Laccaria*, *Lactarius*, *Paxillus*, *Rhizopogon*, *Suillus*, *Thelephora* and *Tylospora* (provided by Drs D. Genney (CEH Merlewood collection), J. Colpaert and D. Mitchell) (Table 1). Fungal cultures were isolated from metal-contaminated habitats (Table 1). These metal polluted sites were (1) the Devon Great Consols copper mine, UK; (2) Niepolomice Forest experimental plots, Poland; (3) Lommel field site, Belgium; and (4) Avoca mine spoil, Ireland. (1) The Devon Great Consols copper mine spoil low-organic soil (southwest England) is highly contaminated with As and Cu. Total (acid-extractable) concentrations of toxic metals in this soil were approximately: 17.1–39.2 mg As g⁻¹ soil, 4.2–13.7 mg Cu g⁻¹ soil and 1.0–1.4 mg Zn g⁻¹ soil (Porter and Peterson 1997; Sharples et al., 2000). (2) The Niepolomice Forest experimental plots were contaminated (in 1980) with up to 5000 tonnes km⁻² y⁻¹ of industrial dusts containing different proportions of Zn, Cd, Al and other metal ions (Greszta et al., 1987). *O. maius* Zn was isolated in 1995 from a plot where Zn was the most abundant metal in the contaminating dust (22.06% ZnO; 0.63% CdO; 8.13% Al₂O₃); whereas Cd was the most abundant toxic metal in the dust (3.02% CdO; 1.75% ZnO; 21.83% Al₂O₃) in the plot where *O. maius* Cd was isolated from (Martino et al., 2000). (3) The EcM isolates from the Lommel Maatheide site were collected in the immediate vicinity of the 'zinc desert', an industrial site polluted with non-ferrous metals emitted by a zinc smelter dismantled in 1973. Toxic metal concentrations in Lommel Maatheide soils were for Zn 1.8 mg g⁻¹ soil; for Cd

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